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PRODUCT INSERT

LIFECODES KIR-SSO TYPING KIT for use with Luminex®

For Research Use Only. Not for use in diagnostic procedures.

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DEFINITION OF SYMBOLS **(Product Labels and Supplemental Documents)**

Batch Code	LOT	Catalog Number	REF	Temperature limitations		Upper limit of temperature	
Use By Date		Keep away from light		Sufficient for N Tests	Σ	Do Not Freeze	
Caution – See instructions for Use		Consult instructions for use		Manufacturer		Danger	

REAGENTS BY CATALOG NUMBER

LM-K LIFECODES KIR Typing Kit for use with Luminex Product # 545110R

Reagent		Product Number	Fill volume	Storage	
MX-K1	LIFECODES KIR-1 Master Mix	545101	170 µL	2-8°C	Σ 20 Sufficient for 20 samples
MX-K2	LIFECODES KIR-2 Master Mix	545201	170 µL	2-8ºC	
BM-K	LIFECODES KIR Probe Mix ‡	545303	720 µL	2-8ºC **Protect From Light**	
DS	Dilution Solution	628155	9.9 mL	18-30ºC	

‡Probe Mixes are light sensitive: keep exposure to light at a minimum.
CAUTION: Do not use components past their expiration date.

SUMMARY AND EXPLANATION

DNA-based KIR typing using PCR amplified DNA is a common laboratory procedure. PCR amplification of DNA is used as the means to enrich for a selected DNA region. For KIR typing, a subsequent assay is utilized to determine the properties of the amplified DNA. Several types of assays, such as SSP (1), direct SSOP (2), and reverse SSOP dot blot technologies, could be used in KIR typing. Like SSOP and reverse dot blot methods, LIFECODES KIR-SSO Typing kits utilize sequence-specific oligonucleotides (SSOs) to identify which KIR loci are present in a PCR amplified sample. It is the set of SSOs employed, not the methodologies that determines the ability to distinguish among the various loci present in the PCR amplification. Whereas reverse dot blot and SSOP methods employ enzyme labels and colorimetric substrates that require subsequent development, the LIFECODES assay is a homogenous multiplex system. That is, all SSOs are analyzed simultaneously and the entire assay is carried out in a single reaction vessel with the addition of a single reagent.

PRINCIPLES OF THE PROCEDURE

The LIFECODES KIR-SSO Typing procedure is based on the hybridization of labeled single stranded PCR product to SSO probes. Amplification of DNA using PCR typically employs equimolar amounts of both forward and reverse primer to generate a double-stranded DNA product. However, if the amount of one primer is in excess relative to the other, the reaction will generate some single-stranded DNA product in addition to double-stranded product. During the initial cycles of the LIFECODES amplification step, double-stranded DNA is generated. Once the limiting primer is exhausted, the remaining primer uses the double-stranded product as a template for generation of single-stranded DNA. This method generates both double stranded and single stranded products that upon denaturation, will both participate in the hybridization reaction.

Each of the different probes may be homologous to a sequence within the amplified DNA that is unique to a locus or group of loci. In other words, these probes are designed so that each probe preferentially hybridizes to a complementary region that may or may not be present in the amplified DNA. In addition, the amplified DNA is also hybridized to one or more Consensus probes homologous to loci present in all samples. SSO Typing can be affected by the type of biological material, method of purification, amount and integrity of genomic DNA. Therefore, the signal obtained for the Consensus probe(s) can serve as an indicator of the success of the amplification and hybridization procedures. Also, the signal obtained with the Consensus probe can be used to normalize the signal of the locus specific probes and correct for variations in the amount of amplified product in the hybridization reaction. The analysis of the results generated from the SSO typing can be used to determine the presence or absence of particular DNA sequences in amplified DNA and to identify the possible loci in the sample.

For the LIFECODES KIR Typing procedure, probes are attached to LuminexMicrospheres designed for use with the Luminex Instrument. Up to 100 different populations of Luminex Microspheres can be mixed together and analyzed by the LuminexInstrument because each population of microspheres can be distinguished by its unique fluorescence signature or color. A different SSO probe can be attached to each color microsphere. Therefore, a mixture of several probes can be distinguished from each other by virtue of their association with particular color microspheres. The Luminex Instrument is also able to quantify the relative amounts of labeled PCR product hybridizing to each Luminex Microsphere. Therefore, the relative signal obtained with the SSO probes in the LIFECODES assay, as with other SSOP methods, can be used to assign the probes as having positive or negative reactivity with the amplified DNA sample (see Results section). This in turn provides the information needed to determine the KIR phenotype of the sample.

REAGENTS

A. Identification

See “Reagents By Catalog Number“ above for complete listing of products and catalog numbers.

B. Warnings or Cautions

1. For Research Use Only. Not for use in diagnostic procedures.
2. Separate pipettes should be designated for Pre-PCR manipulations as well as for Post-PCR manipulations.
3. Biohazard: All biological and blood samples should be treated as potentially infectious. **Use Universal Precautions when handling.**
4. Dilution Solution, Probe Mix, TAQ Polymerase and R-Phycoerythrin Conjugated Streptavidin contain hazardous compounds. Avoid contact with skin and eyes and dispose of accordingly. See Material Safety Data Sheets for additional information.

C. Storage Instructions

1. Refer to kit component packaging label for proper storage temperatures.
2. Probe mixes and R-Phycoerythrin Conjugated Streptavidin are light sensitive, **KEEP FROM LIGHT; DO NOT FREEZE**.
3. Do not use components past their expiration date.

D. Purification or Treatment Required for Use

See “Specimen Collection and Preparation.”

E. Instability Indications

1. If salts have precipitated out of solution during shipping or storage, resolubilize completely prior to use by vortexing at room temperature (18-30ºC).
2. Do not use R-Phycoerythrin Conjugated Streptavidin that has frozen during shipment or storage.

INSTRUMENT REQUIREMENTS

1. Luminex Instrument and XY Platform (Product Number 888300/310)

2. Thermal Cycler equipped with a heated lid, adjustable ramp times, a minimal thermal range of 2°C -100°C and accuracy of at least +/- 0.5°C. Conditions for thermal cyclers may need to be altered in order to optimize the profiles. A thermal profile may be obtained by contacting Technical Service, Tel. (203) 328-9500 or (888) 329-0255.

SPECIMEN COLLECTION AND PREPARATION

A. DNA can be prepared using any preferred method, such as QIAAmp™ (Qiagen) from whole blood, cord blood, stain cards, and buccal swabs. **CAUTION**: **All biological and blood samples should be treated as potentially infectious. USE UNIVERSAL PRECAUTIONS WHEN HANDLING.**

B. DNA extracted from blood preserved in heparin cannot be used in this assay.

C. The isolated DNA should be in 10 mM TRIS, pH 8.0-9.0, or in nuclease free water. If a chelating agent such as EDTA is present the final concentration of the chelating agent should not exceed 0.5 mM.

D. The presence of alcohol, detergents or salts may adversely affect DNA amplification.

E. Final DNA concentration should be 10 – 50 ng/µL.

F. Absorbance measurements of the DNA sample at 260 and 280nm should give a ratio of 1.65 to 2.0.

G. DNA can be used immediately after isolation or stored at –20ºC for up to 1 year. Repeated freeze/thawing should be avoided since this can result in DNA degradation.

PROCEDURE

A. Materials Provided (See tables on page 2 for specific information)

- Master Mix (MX-K1 and MX-K2)
- Probe Mix (BM-K)
- Dilution Solution (DS)
- Threshold Table, Probe Hit Chart(s)

B. Materials, Reagents and Equipment *Required*, but Not Provided (as listed or equivalent)

- R-Phycoerythrin Conjugated Streptavidin (SA-PE), 1mg/mL (Lifecodes Cat No. 628511)
- Luminex Sheath Fluid (1x or 20x, Lifecodes Cat. No. 628005 or 628025)
- Recombinant Taq Polymerase (for kits purchased without Taq)
- Vortex Mixer
- Nuclease-free water (Lifecodes Cat. No. 757003; 20mL)
- PCR tubes and caps (AB Gene® .2ml Thermo Strip, No. AB0451/G)
- Thermal cycler (PCR) 96 well plates (Costar® No. 6509)
- Microseal™ Film (Bio-Rad. No. MSA-5001) or Tape (Costar® No. 6570)
- Thermal cycler (see Instrument Requirements, page 2)
- Bath Sonicator (Fisher Scientific, No. FS15 or FS20)
- Microcentrifuge
- Barrier filter tips
- Luminex Calibration beads (Cal 1, Cal 2, Con 1, and Con 2, Lifecodes Cat. Nos. 628006, 628007, 628008 and 628009 respectively)
- Pipettors, Multichannel pipettors and tips (1-20µL, 20-200µL, 1000µL)
- Spreadsheet software for viewing comma separated value (csv) files
- Heat Block (Fisher Scientific Standard Heatblock, No. 13259-030)
- 70% Isopropanol or 20% Bleach

DIRECTIONS FOR USE

NOTES:

- Probe Mix and SA-PE are light sensitive: **keep away from light and do not freeze**.
- Warm beads at 55º - 60ºC for at least 5-10 minutes to thoroughly solubilize components in probe mixture.
- Sonicate briefly (~15 sec), then vortex probe mix for about 15 seconds to thoroughly suspend the beads.
- Take extreme caution in the aliquoting process, using calibrated pipettes. Failure to do so may result in reagent loss and sample failure.
- All temperatures must be precisely maintained. Fluctuations as little as +/- 0.5°C can affect results.
- At the hybridization stage, samples should not remain in the diluted state at 56°C for more than 5 minutes (see Results section).
- It is recommended to assay the amplified samples as soon as possible. If the samples can not be run on the Luminex Instrument the same day, ***the amplified product can be stored up to 3 days at 2-8ºC prior to use. For longer storage, store at –20ºC up to one week until ready to assay. The amplified product can only be frozen and thawed once. Repeated freezing and thawing will result in degradation of amplified samples and will yield poor results if assayed***.
- It is recommended that one negative and positive control be run with each test. SSO probes that react with loci present in all individuals are used as Consensus Probes. Values obtained with the Consensus SSOs from positive controls should exceed the threshold value for the SSO as set forth in the Threshold Table Worksheet.

A. Purify genomic DNA, using method of choice; final concentration should be 10 – 50 ng/µL. Adjust, if necessary, with nuclease free water. Keep all samples at similar concentrations.

B. DNA amplification (PCR)

1. Allow the Master Mix for the appropriate loci to warm to Room Temperature (18-30ºC).
2. Gently vortex the reagents for approximately 10 seconds. This will ensure the salts are in solution. Spin briefly (5 – 10 seconds) in microcentrfuge to bring contents to the bottom of the tube.
3. The multiplex amplification of the KIR loci is divided into two amplification reactions that utilize the KIR-1 Master Mix (MX-K1) and the KIR-2 Master Mix (MX-K2).
4. Using **Table 1** below, prepare the components for amplification for n+1 samples using the indicated amount of each component per reaction (except for DNA). Bring to a final volume of 20µL per reaction with nuclease free water. Gently vortex, spin down and place on ice.
5. Pipette the appropriate amount of Genomic DNA **(~50ng)** into the PCR tubes.
6. Aliquot the amplification mix into the PCR tubes containing the genomic DNA. (The total volume of amplification mix and genomic DNA should equal 20µL for each sample reaction.)
7. Cap tubes tightly to prevent evaporation during PCR.
8. Place samples in the thermal cycler and run program. See **Table 2.**

Table 1. Reaction Components for Amplification

Component	Amount per PCR sample reaction
LIFECODES Master Mix (MX-K1 or MX-K2)	6 µL
Genomic DNA **10-50ng/µL**	**Total of ~50ng**
Taq polymerase	0.2µL (1.0U)
Nuclease-free water	To 20µL final volume

Table 2. Thermal Cycler Conditions for Amplification

Step	Temperature and Incubation Time	# of Cycles
1	95º C for 2 min	1
2	94º C for 30 sec	40
	59º C for 90 sec	
	72º C for 30 sec	
3	72º C for 15 min	1
4	4º C forever	

Note: To be sure of sample amplification, refer to Product Gel Electrophoresis (Appendix A).

C. Hybridization

- **Be sure hybridization buffer components of the LIFECODES Probe Mix are solubilized and that the beads are thoroughly suspended.**
- **Turn on the Luminex Instrument and XY Platform to allow for 30 minute warmup.**

1. Warm Probe Mix in a 55º - 60ºC heat block for at least 5-10 minutes to thoroughly solubilize components in probe mixture.
2. Pre-warm the thermal cycler to be used for hybridization to 56°C.
3. Sonicate briefly (~15 sec), then vortex Probe Mix for about 15 seconds to thoroughly suspend the beads.
4. Aliquot 5 µL of locus specific PCR product into a thermal cycler 96 well plate (Costar® No. 6509).
5. Aliquot 15 µL of Probe Mix into each well. When aliquoting probe mix to more than 10 wells, gently vortex Probe Mix after each set of ten. Seal plate with Microseal® film.
6. Hybridize samples in the pre-warmed thermal cycler at 56°C (56°C HOLD) for 20 minutes.
6. While the samples are hybridizing, prepare a 1:200 Dilution Solution/SA-PE mixture. Combine 170µL Dilution Solution (DS) and 0.85 µL 1mg/mL SA-PE per sample. *It is recommended to make enough Dilution Solution Mixture for n+1 samples to account for pipetting loss.* ***(See Table 3)***
7. *Keep Dilution Solution/ SA-PE mixture in the dark, at room temperature; SA-PE is light sensitive!* The Dilution Solution may be warmed at 45ºC for 5 minutes and vortexed upon arrival to ensure all components are in solution. Dilution solution must be at room temperature before making the mixture**. Prepare prior to use and discard any remaining portion.**

Table 3. Dilution Solution Preparation Volumes

# of Samples	Dilution Solution (DS)	SA-PE
1	170µL	0.85µL
5	850µL	4.25µL
10	1700µL	8.5µL
20	3400µL	17µL
50	8500µL	42.5µL

Note: DO NOT CANCEL HYBRIDIZATION PROGRAM BEFORE REMOVING THE TRAY FROM THE THERMAL CYCLER!

8. After the hybridization period, while the tray is still on the thermal cycler at 56ºC, dilute each sample with 170µL of the prepared Dilution Solution/ SA-PE mixture.
9. Remove the sample tray from the thermal cycler and place in the Luminex Instrument.

D. Analyze sample using the Luminex Instrument*

For best results, assay the samples immediately using the Luminex Instrument. Samples can be read up to 30 minutes after being diluted. If not read immediately, protect samples from light.

1. Turn on the Luminex Instrument between 30 minutes and 4 hours before assaying the samples.
2. Prior to analyzing the samples on the Luminex Instrument, set up a Batch Run by which the samples will be analyzed.
   a) Select ***Create a New Batch*** from the File menu.
      - Add Batch for KIR-1. Do the same for KIR-2.
      - The Batch Templates are provided and are named KIR-1 and KIR-2.
      - Please note that the template versions are lot number specific and correspond to the kit lot numbers.

- Follow the stepwise instructions that appear on the screen for creating batches.
- **When naming the batch, do not include commas in the name because information after a comma will be lost upon exportation of the data.**
- For further instructions on creating batches and multibatches, refer to the Luminex IS User's Manual

b) Click the eject icon to eject the plate holder. Place the 96 well thermal cycler plate containing the samples in the XYP heater block present on the plate holder.
c) Click the Retract icon. The samples are now ready to be analyzed. A prime step should be performed before starting the run.
d) After the samples have been run through the instrument, a sanitization step with 70% Isopropanol or 20% household bleach should be performed followed by two wash steps. The instrument can be turned off at this point if it is not going to be used for the remainder of the day.

3. After a batch is complete, the data is exported as a comma separated values (csv) file. These files are named 'OUTPUT.CSV' and saved in a folder with the Batch Name entered into the Luminex IS. This data is then available for making typing assignments as described below.

*****Refer to Luminex IS User's Manual for instrument operation, including daily startup, calibration, maintenance, and shutdown procedures.

RESULTS

Sample typing can be done as follows:
The generated CSV files can be opened and the data processed with common spreadsheet programs such as Microsoft Excel, Lotus 123, Corel Quattro Pro, or similar software. Analysis is comprised of the following steps:

1) Verify that the Number of Events for each SSO in each sample is at least 60. This information is found in the ***DataType: Count*** section of the CSV file.
2) Determine that the values for the Consensus probes for each sample are above their minimum Median Fluorescent Intensity or MFI. The minimum thresholds are lot specific and can be found in the Threshold Table.

Caution:

- *To obtain reliable results, there must be sufficient data gathered by the Luminex Instrument.*
- *Collect at least 60 events for each SSO.*
- *The LIFECODES Probe Mix contains two SSOs (designated CON100 and CON200) that hybridize to KIR locus 3DP1and 3DL3 respectively. These act as internal controls to verify that the PCR reactions and hybridizations worked.*
- *If the minimum value is not obtained for these SSOs, the sample may not produce the correct typing and the sample test should be repeated*.

3) Subtract the *Background Control* value for each probe from the sample values producing the background corrected data set. Background Control values are found in the Threshold Table and are lot specific. Background values are average MFI values for each bead to compensate for background noise due to bead variation.
4) For each sample, divide the background-corrected data for each probe by the background-corrected value for the corresponding consensus probe producing the normalized data set.

$$\frac{\text{MFI (Probe)} - \text{MFI (Control blank for probe)}}{\text{MFI (Consensus)} - \text{MFI (Control blank for consensus)}}$$

5) For each probe, record the normalized value on the Threshold Table Worksheet.
6) Once all values have been assigned, the probe hit pattern (i.e., the combination of all positive and negative assignments for a given sample) can be compared with the Probe Hit Table provided.

Caution:

- *There is a separate threshold table for each locus.*
- ***These threshold tables are Lot-specific; be certain that the Lot # on the threshold tables matches the Lot # of the typing kit.***
- *If a normalized value for a particular probe falls above the maximum threshold for a negative assignment and below the minimum value for a positive assignment, the sample should be considered as indeterminate for this probe.*
- *If desired, this probe can be removed from consideration or the sample can be typed, first assuming the value to be negative and then again assuming the value can be positive.*
- *See **EXPECTED VALUES** section for further information on threshold values.*

LIMITATIONS OF THE PROCEDURE

The PCR conditions and assay conditions described require precisely controlled conditions. Deviations from these parameters may lead to product failure.

All instruments must be calibrated according to manufacture's recommendations and operated within manufacture's prescribed parameters.

1) Beads must be pre-warmed and well suspended prior to use. This ensures that the hybridization buffer components are in solution.
2) The 56ºC incubation requires a high degree of accuracy (+/- 0.5ºC). A thermal cycler should be employed. Temperature should be verified, within wells of the 96 well thermal cycler plate, using a thermocouple (e.g., Bio-Rad, Model VPT-0300 or equivalent). The temperature within wells and among wells should not vary more than +/- 0.5ºC.
3) Time at 56ºC is critical and should not exceed a total of 25 minutes. This includes the 20-minute incubation plus no more than 5 minutes to dilute all the samples with Dilution Solution/ SA-PE mixture.
4) Once diluted, the samples are stable at room temperature for up to 2 hours (protect from light). Since a full 96-well plate can take up to 1.5 hrs to run through the Luminex Instrument, the analysis should be started no more than 30 minutes after dilution to ensure that the last sample is analyzed within the 2 hr limit.
5) Do not mix components from other kits and lots.

Due to the complex nature of KIR typing, qualified personnel should review data interpretation and typing assignments.

TROUBLESHOOTING

PROBLEM	POSSIBLE CAUSE		SOLUTION
Low Bead Count	Probe Mix not well suspended		Prewarm, sonicate and vortex Probe Mix and repeat assay.
	Instrument not functioning properly	Out of Calibration	Calibrate Instrument. *(Refer to Luminex IS User's manual.)*
		Sample flow path blocked	Remove and sonicate needle. Perform backflush. Call Immucor Transplant Diagnostics, Inc. if problem persists. (888) 329-0255
CON Threshold Failure	Sample failed to amplify or amplified poorly*	Low DNA	Check DNA concentration and purity.
		Salts in Master Mix are out of solution	Heat the Master Mix at 37ºC for 5 minutes, vortex gently and spin down briefly.
		Poor Taq Polymerase	Use only Recombinant Taq. Try Lifecodes # 167075.
	Amplification conditions not within specific parameters		Run thermal profile on thermal cycler to verify parameters are within specified parameters.
	Low Median Fluorescent Intensity Value (MFI)		Warm dilution solution at 45ºC for 5 minutes before use and vortex. Store at room temperature.
			Replace R-Phycoerythrin Conjugated Streptavidin.
Multiple SSO failures or sample fails to yield a KIR typing result	Locus specific amplification	Amplification conditions not within specific parameters	Run thermal profile on thermal cycler to verify parameters are within specified parameters.
	DNA sample contaminated		Re-isolate DNA from blood sample.
	DNA partially degraded		
	Evaporation during hybridization step		If not using an entire plate, leave one row empty on each side of samples to be assayed to allow plate to be sealed tightly.

*** PCR amplification can be verified by gel electrophoresis (See Appendix A).**

EXPECTED VALUES

Values can fall into three ranges: Negative, Positive, or Indeterminate. An "indeterminate value" represents a range in which neither positive nor negative values have been observed. If a sample contains indeterminate values for a particular SSO probe, the sample should be re-assayed for confirmation. It may also be necessary to re-isolate DNA from the sample and re-amplify and re-assay.

As noted in the ***Limitations of the Procedure*** section, it is critical to precisely follow the protocol. Any deviations can lead to sample typing failure.

SPECIFIC PERFORMANCE CHARACTERISTICS

Specific performance characteristics are to be determined.

REFERENCES

From SUMMARY AND EXPLANATION Section:

1. Hsu,K., et al. (2002) The Journal of Immunology 169: 5118
2. Crum, KA. et al. (2000) Tissue Antigens 56: 313

General KIR References:

Vilches,C and Parham,P (2002) Ann.Rev.Immunol. 20: 217

Marsh, SGE, et al. (2003) Human Immunology 64: 648

LIMITED LICENSE

This product is not supplied with Taq polymerase and does not include a license under the foreign patents, owned by F. Hoffmann-La Roche Ltd. and Roche Molecular Systems, Inc to practice the Polymerase Chain Reaction ("PCR") process described in said patents. Further information on the need to obtain purchasing licenses in your country may be obtained by contacting F. Hoffmann-La Roche Ltd. or Roche Molecular Systems, Inc. The purchase of this product includes a limited, non-transferable license under U.S. patent 5,981,180 or its foreign counterparts, owned by Luminex Corporation, to perform multiplex analysis of clinical specimens for KIR typing.

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TRADEMARKS USED

AB Gene®	AB Gene House	QIAAmp™	Qiagen Inc.
Costar®	Corning Incorporated	GelStar®	Cambrex Bio Science
Microseal™	Bio-Rad Laboratories, Inc.		
IDNA® Agarose	Cambrex Bio Science		
Luminex®	Luminex Corp.		

APPENDIX A

Gel Electrophoresis

The PCR reactions performed in the LIFECODES KIR Typing Kits are designed to produce both double and single stranded products, which are the predominant products that hybridizes to the SSOs. For quality assurance or to trouble shoot an experiment it might be necessary to perform gel electrophoresis to examine the PCR reaction for the presence of amplified DNA.

Materials Required (as listed or equivalent)

- Electrophoresis Grade Agarose (Cambrex IDNA® Agarose No. 50170)
- Electrophoresis apparatus/power supply
- 1X Gel Buffer (40xTAE, Promega No. V4281)
- GelStar® Nucleic Acid Gel Stain (Cambrex No. 50535)
- UV Transilluminator (ChromatoVUE, UVP Inc. Model TM36)
- Photographic imaging system

The relative migration of the single stranded product is dependent upon the gel concentration and buffer system employed. Approximate migrations for each amplification are listed below for samples run in a 2% Agarose gel in 1X TAE buffer.

Electrophoresis Conditions

1. Remove GelStar® Nucleic Acid Stain (Cambrex No 50535) from freezer to thaw. Keep in dark.
2. The gel used for this procedure must be 2%, i.e. for a 200ml gel bed use 4 grams of agarose to 200mL 1X TAE (Dilute from 40X TAE). Add 10µL GelStar® Nucleic Acid Stain to the molten agarose. When pouring the gel be sure to leave ample room for DNA to run a significant distance (1 to 2 inches). **USE CAUTION: GelStar® is a potential Carcinogen.**

NOTE: It is possible to run gels with 20µL of 10mg/mL Ethidium Bromide in place of GelStar® Nucleic Acid Stain. Product band intensity will be less in gels containing Ethidium Bromide than in gels containing GelStar®. **USE CAUTION: Ethidium Bromide is a known Carcinogen.**

3. Keep gel in dark and allow to solidify.
4. Load a mixture of 2.5µL of each PCR product and 2.5µL 2X loading buffer with visible dye per sample, per amplification. Let gel run in the dark at approximately 160 volts for 45 minutes or until sample runs far enough to see separate bands for single and double stranded product (bromophenol blue band or other visible marker migrates 1 to 2 inches from wells).
5. Photograph using UV Transilluminator accompanied by a GelStar® Yellow Photographic Filter (Cambrex No 50536).

CAUTION: Wear protective equipment when handling GelStar® Nucleic Acid Stain or Ethidium Bromide and when photographing gel using UV Transilluminator.

6. Gel analysis

	KIR-1	KIR-2
DNA Fragments (bp)	~250	~250, ~800

Gel Interpretation